A PHASE 1/2 TRIAL OF A TETRAVALENT LIVE-ATTENUATED DENGUE VACCINE IN FLAVIVIRUS-NAÏVE THAI INFANTS

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ABSTRACT

The Walter Reed Army Institute of Research (WRAIR) has produced a tetravalent live-attenuated dengue vaccine that has been well tolerated and immunogenic in U.S. adults and Thai children. As infants are considered by many as an important age group for vaccination in dengue-endemic countries, we evaluated the vaccine in Thai flavivirus-naïve infants who are at risk for dengue.

Fifty-one healthy flavivirus-naïve infants aged 12-15 months were enrolled and randomly assigned to one of two groups at the Phramongkutklao Hospital of the Royal Thai Army. Group I (N=34) received dengue vaccine at study months 0 and 6; Group II (N=17) received control vaccines (varicella at study month 0; Hemophilus influenza B at study month 6). All received a licensed inactivated Japanese encephalitis (JE) vaccine at study months 7 and 7.5. Solicited and unsolicited adverse events were collected for 21 and 31 days, respectively, after each vaccination; serious adverse events (SAEs) were collected throughout the study. Safety testing included complete blood count and liver enzymes measured at intervals after each vaccination. Antibody endpoints were determined by 50% plaque reduction neutralization test using each serotype of dengue virus.

Fifty infants completed all study visits; one infant was withdrawn due to re-location remote from Bangkok. All infants tolerated the vaccinations without SAEs attributed to vaccination as reported by an independent data monitoring committee. Two infant recipients of dengue vaccine experienced one day of grade 3 fever, one with a maximum temperature of 39.2°C occurring 19 days post-dose 1 of dengue/control vaccination and the other with 40.2^0C occurring 5 days post-dose 1 vaccination. The seroconversion rates among those receiving 2 doses of undiluted vaccine (according to protocol cohort) were 55.2% (Den1), 100% (Den2), 86.2% (Den3), and 96.4% (Den4).

The WRAIR tetravalent live-attenuated dengue vaccine immunized against 4 dengue virus types and was well tolerated in this preliminary infant trial.

1. INTRODUCTION

The basis of dengue as a major threat to deployed troops lies in the fact that this mosquito-borne virus has been systematically expanding within the subtropics and tropics where 2.5 billion people reside resulting in an estimated 50-100 million new infections annually (WHO, 1997; Gubler, 1998) Thailand, hyperendemic for dengue, has experienced large outbreaks of disease occurring every three years (Cummings et al., 2004).

Currently, no specific anti-viral therapy exists and vector (mosquito) control and personal protective measures have failed to provide long-term solutions to deployed troops (Halstead, 1984; Gubler, 1997; Ooi et al., 2006). Vaccination offers a reasonable alternative as has been successfully achieved against related viral diseases

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e.g., yellow fever and Japanese encephalitis (Stephenson, 1998). Prevention of dengue through vaccination has become a major priority on the agendas of World Health Organization, national ministries of health and military organizations, especially in countries endemic for dengue e.g., Thailand (Brandt, 1990).

Dengue viruses belonging to the family Flaviviridae and the genus Flavivirus, consist of 4 distinct serotypes: DEN-1, DEN-2, DEN-3 and DEN-4. These viruses cause both dengue fever (DF), but also the more severe form of the infection termed dengue haemorrhagic fever (DHF) (Gubler, 1997).

Dengue viruses are transmitted to humans principally by the Stegomyia (Aedes) aegypti species mosquitoes. Dengue virus infections can result in DF (recognized for >200 years), a self-limited but incapacitating acute illness lasting four to seven days characterized by fever, headache, rash, severe muscle, joint and eye pain. Dengue can be further complicated by the development of DHF (recognized since 1950's) manifest by plasma leakage and/or bleeding diathesis or frank hemorrhage with fatality of 1-5% (WHO, 1997; Gubler, 1998). Soldiers exposed to sequential dengue virus infections with multiple dengue virus serotypes are at risk for DHF given that the antibodies from an earlier dengue infection with one serotype places that individual at a higher risk for developing DHF upon subsequent exposure to another dengue virus serotype (Halstead 1970; Avirutnan et al., 2006). As the four dengue virus serotypes spread, the number of outbreaks of DHF increase.

The U.S. Army has developed a live-attenuated tetravalent dengue virus vaccine consisting of a combination of each of the four dengue virus serotypes that have individually been serially passaged in primary dog kidney (PDK) cells to achieve attenuation. Each PDK-passaged virus strain was prepared as a master seed, working seed and vaccine bulk by three additional passages in fetal rhesus lung (FRhL) cells. Monovalent vaccines of varying potency are reconstituted and combined to make various formulations of tetravalent dengue vaccine (TDV).

In a series of phase I studies conducted by the U.S. Army beginning in 1989 and ending 2002, six attenuated DEN strains were identified for the tetravalent combination vaccine. Phase I studies conducted in a small number of adults indicate that Formulation 17 (F17) has improved immunogenicity and acceptable reactogenicity compared to other tested formulations (Edelman et al., 2003, unpublished data).

A small phase I/II trial of the same tetravalent formulation (F17) was conducted in flavivirus antibody naïve children aged 6-9 years in Thailand. Data from this study indicated that the vaccine was well tolerated and immunogenic in this study population. All six subjects (100%), in the ATP immunogenicity cohort had a tetravalent N antibody response one month after the second dengue vaccine dose (Simasathien S, unpublished data).

Given that susceptibility to dengue begins in infancy as maternal antibody wanes, the presumption has been that the optimal age for vaccination is soonafter maternal antibody wanes, i.e., after 12 months of age. Consequently, this study aimed to evaluate both safety and immunogenicity in the pediatric population, specifically those 12-15 months of age. The main objectives of this study were to compare the reactogenicity of this formulation (F17) in terms of solicited symptoms within the 21-day follow-up period after dose 1 of the dengue vaccine and control vaccine (Varicella vaccine) and to assess the immunogenicity of the dengue vaccine at 30 days after the 2-dose series.

2. MATERIALS AND METHODS

2.1 Study subjects

Parents of healthy infants aged 12-15 months gave informed consents to have their infants' blood screened for antibodies to dengue and Japanese encephalitis (JE) virus, anemia, neutropenia, thrombocytopenia, abnormal AST and ALT. Flaviviral antibody naïve infants with normal screening laboratory tests were included in the enrollment process. Exclusion criteria included chronic administration of immunosuppressants, recent recipient of blood products, acute moderate to severe illness at time of vaccination, positive HBsAg, anti-HIV or anti-HCV.

2.2 Study design

This randomized, double blind, controlled trial was conducted in the Phramongkutklao Hospital, Bangkok, Thailand. The protocol and consent forms received extensive review and final approval by the ethical review committees of the Thai Ministry of Public Health (MoPH), the Royal Thai Army and the U.S. Army (Human Subjects' Research Review Board). Two doses of live-attenuated tetravalent dengue vaccine F17 were given 6 months apart. Varicella vaccine and Hib vaccine were used as control vaccines for dose 1 and dose 2, respectively. Two doses of JE vaccines were given at months 7 and 7.5 of the study. Volumes of 1.0 mL for dengue vaccine or 0.5 mL for control vaccines were delivered in the lateral aspect of the middle third of the infant's thigh by an independent vaccination team. Ninety-four infants were screened in order to enroll 51 healthy flavivirus antibody naïve infants. The ratio of study group to control group was 2:1. Thirty-four

flavivirus-naïve infants were randomized to receive dengue vaccine and 17 to receive control vaccine. Three cohorts of 6, 15 and 30 subjects were sequentially vaccinated with interval safety data reviewed by an independent Data Safety Monitoring Board (DSMB) and the U.S. Food and Drug Administration (US FDA). The dengue vaccine recipients of the first cohort received one-tenth dose of dengue tetravalent vaccine and those of the remaining cohorts received full dose. Approval by the DMC permitted continuation of the study to include administration of a second dengue vaccine dose and to start the next cohort.

2.3 Vaccine composition

DEN candidate vaccine: The tetravalent, live attenuated DEN vaccine candidate, F17, was used in this study. This formulation contains dengue serotype 1, 2, 3 and 4 vaccines that were produced at the WRAIR Pilot Bioproduction Facility, Silver Spring, Maryland. One dose of dengue vaccine formulation 17 contains 1 mL of dengue vaccine consisting of DEN-1 (45AZ5, PDK 27), DEN-2 (S16803, PDK 50) and DEN-3 (CH53489, PDK 20) and DEN-4 (341750, PDK 6). This formulation contains 50 mcg/mL neomycin base, 5.5% lactose, and 1.9g% human serum albumin; for subcutaneous injection.

JE vaccine: The licensed JE vaccine was produced by the Thailand GPO using a Beijing strain of JE, in liquid form, dosed at 0.5 mL for subcutaneous injection.

2.4 Safety and reactogenicity

Participating infants were evaluated for local solicited symptoms, general solicited symptoms, serious adverse event, other adverse event, alert laboratory values and vaccine viremia. After each vaccination the subjects were observed for 30 minutes to detect any immediate reaction. Then the reaction to vaccines were assessed by a combination of daily solicited symptom diaries maintained by the infants' parents daily for 21 days after each dengue/control vaccination and 7 days after each JE vaccination, unsolicited events to be recorded up to 30 days of each dengue/control vaccination, by telephone reports to the research nurse, by scheduled visits to the study physician and by all unscheduled visits. The solicited and unsolicited symptoms were recorded in the diary card. Parents were instructed in taking axillary temperatures using digital thermometers every evening for 21 days following each dengue/control vaccination. Temperatures were graded as grade 0 (<37.5^{o}C), grade 1 (> 37.5^{o}C and < 38^{o}C), grade 2 (>38^{o}C and < 39^{o}C) and grade 3 (>39C^{o}). Redness or swelling were also measured by the parents; this led to grading as follows: grade 0 (none), grade 1 (greatest surface diameter <5 mm.), grade 2 (5-20 mm.) and grade 3 (>20 mm.). Pain was graded as grade 0 (none), grade 1 (minimal reaction to touch) grade 2 (the child cries or protects on touch) and grade 3 (the child cries when the limb is moved or spontaneously painful). Other systemic adverse events were classified as grade 0 (none) grade 1 (minimal discomfort), grade 2 (interferes with normal daily activities) and grade 3 (prevents normal daily activities).

Telephone contacts were performed to inquire about the infants' health during period between clinic visits. Any illness reported by the guardian would prompt an unscheduled visit and evaluation by the study physician. Serious adverse events including untoward medical occurrence that resulted in death, life threatening condition, hospitalization or prolongation of existing hospitalization or disability were assessed throughout the entire study. Alert laboratory values including absolute neutrophil count (ANC) of less than $1x10^{9}$/L, platelet count $<100x10^{9}$/L, AST or ALT values greater than 2.5 times of normal upper limits for age from blood samples drawn on days 10 and 30 of each vaccination were monitored and followed until returning to normal. Presence of vaccine- induced viremia to any dengue serotype was performed by RT-/nested PCR on day 10 of each dose of DEN/control vaccination. Emergency room visits, outpatient clinic visits, physician office visits unrelated to routine examination, vaccination or common illnesses were reported throughout the period of the study.

2.5 Laboratories

Safety laboratory tests: Complete blood count including differential counts, AST and ALT were performed in an accredited commercial laboratory in Bangkok, Thailand.

Serology for Dengue and JE viruses: Screening assays: HI and $PRNT_{50}$ vs. DEN-1, -2, -3, -4 and JE were used to test serum at 1:10 dilution for Flavivirus naïve infants. Endpoint assay: Immunogenicity endpoint was performed by $PRNT_{50}$ using 4-fold dilutions of serum tested. Antibodies to dengue and JE viruses were measured by hemmaglutination inhibition (HI) and plaque reduction neutralization test (PRNT) at AFRIMS or WRAIR. The assay procedures were performed according to the previous reports. For HI, a dilution of 1:10 was tested for the presence of each of DEN serotype and JE HI antibodies as previously described with modification to a microtiter plate format (Clarke and Casals, 1958). For PRNT, a dilution of 1:10 was tested for the presence of DEN 1-4 and JE (Nakayama) neutralizing antibodies in the screening process and 50% PRNT titer against all DENV serotypes and JEV as the end point determination for the vaccine immunogenicity (Russell and Nisalak, 1967). Complement was not added to the neutralization mixture.

Virus detection and genome sequencing: Virus detection for DENV was performed by nested PCR using TaqMan probe and real time PCR. Virus RNA was extracted from serum using QIAamp viral RNA mini kit (QIAGEN, Germany) according to the manufacture's instructions. The extracted RNA was reverse transcribed and first round DNA amplified in the same tube. The nested PCR was done in the second round PCR by using TaqMan probe and real time PCR machine. All primers and probes were universal binded to both natural and vaccine viral strains. Detection of fluorescent signals exponentially for 3 consecutive cycles were determined as positive for virus presence. All samples positive by virus detection were sequenced for envelope (E) gene on a MegaBACE 500 automated DNA sequencer (Amersham Pharmacia Biotech). Overlapping nucleic acid sequences were combined for analysis and edited with the aid of Sequencher software (Gene Code Corp.) to distinguish viremia from natural infection and viremia induced by the vaccines.

3. RESULTS

The detailed results of this study will be submitted to an international peer-review journal for potential publication. We provide here a brief synopsis of the results.

The numbers of subjects in study group/control group were 4/2, 10/5 and 20/10 in cohorts A, B and C, respectively. One control subject in cohort C withdrew from the study after receiving the first dose of varicella vaccine due to relocation remote from Bangkok. One subject became infected by wild-type DEN-4 virus approximately 90 days after dose 1. These 2 subjects were included in the safety analysis but were eliminated from the ATP immunogenicity analysis. The enrolled infants ranged from 12-15 months of age at dose 1 vaccination, 54.9% were males and all were of Southeast Asian (Thai) descent.

Safety analysis was performed on the total vaccination cohort. Analyses were performed for each cohort (A, B, C) and for pooled cohorts B and C. There were no immediate adverse events during the 30-minute observation period immediately following each vaccination and no withdrawal due to an adverse event throughout this study. For local solicited symptoms after dose 1, pain, redness and swelling were more frequently reported in study group than control group. There was no significant difference in local AE between low dose (1/10) and full dose groups. Redness was the most frequently reported local reaction, typically lasting 1 day and none longer than 3 days.

There was no major difference in the occurrence of solicited general symptoms and unsolicited symptoms in study group and control group. Fever was reported more frequently after full dengue dose 2 than dose 1 (26.7% after dose 1 and 53.3% after dose 2) however the occurrence of fever was not different from the control group (47.1% after dose1 and 50% after dose2). Grade 3 fever (temp > 39^0 C) was reported in 5% of full dose dengue vaccine group and 6.1% of control group. The most frequent finding upon physical examination was lymphadenopathy not distinguishable from baseline. There were 4 serious adverse events, all due to hospitalization, but none related to vaccination.

Immunogenicity: None of the subjects who received control vaccines developed seropositivity for any serotype of DEN N antibodies throughout the study period. One month after the second dose in the full dose dengue vaccine cohorts (B and C), 55.2% were seropositive for DEN-1 N antibody, 100% for DEN-2, 86.2% for DEN-3 and 96.4% for DEN-4 N antibody. The $1/10^{th}$ dose dengue vaccine provided inferior immunogenicity profile.

4. DISCUSSION

Deployed U.S. troops require a dengue vaccine as alternate measures of vector control and/or personal protective measures have been impractical. In order for the U.S. Army to enlist a pharmaceutical partner to produce a dengue vaccine, licensure must be sought, not only in the adult population, but also in the pediatric population that suffers the greatest morbidity and mortality related to this disease. Consequently, this study demonstrating safety in this infant population was critical to sustaining the clinical development plan of this vaccine towards U.S. FDA licensure and utilization by our U.S. forces.

Dengue vaccination and control vaccination appeared indistinguishable, except for local reactogenicity. The lower dose (1/10 of the full dose) of dengue vaccine was not associated with a better safety profile and lower reactogenicity. This suggests that it is the full dose that should be further studied given its greater potential to provide better immunogenicity without compromising the safety profile of the product. The cohort effect observed with respect to immunogenicity in the cohorts that received the full dose of dengue vaccine requires further exploration in larger studies.

CONCLUSION

The investigational full dose dengue vaccine (F17) was well-tolerated and immunogenic in healthy infants in Thailand who lacked prior flavivirus antibody.

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